DER Addendum For 28-Day Whole Sediment *Leptocheirus plumulosus* Toxicity Test: (Original Study MRID: 46591501; Amended Report MRID: 48762901)

Amended Report Citation:

Authors: Putt, A.E.
Title: Bifenthrin - Toxicity to Estuarine Amphipods *(Leptocheirus plumulosus)* During a 28-Day Sediment Exposure. Amended Final #2
Study Completion Date: 15 February 2012
Laboratory: Springborn Smithers Laboratories, 790 Main Street, Wareham, MA 02571-1037
Sponsor: Pyrethroid Working Group, Beveridge & Diamond, 1350 I Street NW Washington, DC 20005
Laboratory Report ID: 13656.6107
MRID No.: 48762901

Findings:

The original 28-d whole sediment toxicity study of bifenthrin to the estuarine amphipod, *Leptocheirus plumulosus* was classified as supplemental due to the absence of reproduction data (MRID 46591501). The registrant submitted an amended study report which contained the raw and summarized reproduction data (See attached Table 10 from the amended study report). This reviewer analyzed the reproduction data using CETIS v. 1.9.1. According to the Shapiro-Wilk W Normality test, reproduction data conformed to a normal distribution (α =0.01) but variances were not homogeneous among treatments (Bartlett Equality of Variance Test, α =0.01). Therefore, all subsequent statistical analysis relied on nonparametric tests. The solvent and negative control mean # of offspring did not differ statistically. Since the solvent is evaporated from the spiked sand prior to addition to test sediments, a solvent effect would not be expected. Furthermore, due to a significant reduction in amphipod survival at 130 µg ai/kg-dw (mean measured), statistical results for sublethal effects on amphipods in this treatment are considered unreliable due to potential bias in the results for more tolerant individuals.

Data were first analyzed relative to the negative control using the Mann-Whitney U Two-Sample Test and the Jonckheere-Terpstra Step-Down Test. Both tests indicated significant reductions ($p<0.05$) in mean # offspring/amphipod at 50 µg ai/kg-dw (nominal), but not at the lower treatments (5.4 and 20 µg ai/kg-dw) despite a 53% - 62% reduction in offspring relative to negative controls. This low statistical power was likely due in part to the high variance associated with the negative control (CV = 87%) and relatively low sample size (n=5/treatment).

Mean Meas. Conc. µg/kg sed	Control Code	n	Mean # Offspring/ Amphipod	Median	Min	Max	Std Err	CV%	%Effect
0	Neg	5	9.92	5.7	2.2	22.6	3.873	87.31%	0.00%
5.4		5	3.74	3.6	1.8	7.2	0.9958	59.54%	62.30%
20		5	4.64	4.6	1.5	8.2	1.073	51.69%	53.23%
50		5	2.58 *	1.2	1	6.1	0.9957	86.30%	73.99%
130		5	0.52 * ¶	0.4	0.3	1.1	0.1463	62.91%	94.76%

* significantly reduced relative to negative control, Mann-Whitney U and Jonkheere-Terpstra Step Down Test, $p<0.05$)
¶ significant reduction in amphipod survival in this treatment.

In order to increase statistical power, controls were pooled and the data were re-analyzed. Results from both non-parametric tests indicated statistically-significant reductions in mean # of offspring at all treatment levels relative to the pooled control.

Mean Meas. Conc. µg/kg sed	Control Code	n	Mean # Offspring/ Amphipod	Median	Min	Max	Std Err	CV%	%Effect
0	Pooled	10	12.05	12.9	2.2	22.6	2.127	55.83%	0.00%
5.4		5	3.74 *	3.6	1.8	7.2	0.9958	59.54%	68.96%
20		5	4.64 *	4.6	1.5	8.2	1.073	51.69%	61.49%
50		5	2.58 *	1.2	1	6.1	0.9957	86.30%	78.59%
130		5	0.52 * ¶	0.4	0.3	1.1	0.1463	62.91%	95.68%

* significantly reduced relative to negative control, Mann-Whitney U and Jonkheere-Terpstra Step Down Test, p<0.05)
¶ significant reduction in amphipod survival in this treatment.

Study Conclusions:
In considering these additional reproduction data, the overall (most sensitive) NOAEC and LOAEC values from this study are as follows.

Based on mean measured concentrations in sediment dry weight:

NOAEC: < 5.4 µg ai/kg-dw
LOAEC: 5.4 µg ai/kg-dw

Based on mean measured concentrations normalized to sediment organic carbon:

NOAEC: < 132 µg ai/kg-OC
LOAEC: 132 µg ai/kg-OC

Based on ESTIMATED[1] concentrations in sediment pore water:

NOAEC: < 0.6 ng ai/L pore water
LOAEC: 0.6 ng ai/L pore water

Most sensitive endpoint: amphipod reproduction

Study Classification: This study is considered scientifically sound but is classified as SUPPLEMENTAL and is acceptable for quantitative use in risk assessment.

Rationale: a definitive NOAEC was not reached in this study.

Reparability: none.

Primary Reviewer: Keith G. Sappington, Senior Science Advisor, USEPA/OPP/EFED/ERB5

Signature: **Date:** 8/10/2016

Secondary Reviewer: Justin Housenger, Biologist, USEPA/OPP/EFED/ERB5

Signature: **Date:** 8/10/2016

[1] Pore water concentrations estimated from sediment dry weight concentrations using a mean Koc of 236,800 L/kg-OC and 4.1% sediment TOC.

From the Study Report:

Table 10. Mean percent survival, mean dry weight and mean number of offspring/amphipod at test termination of the 28-day exposure with bifenthrin and amphipods (*Leptocheirus plumulosus*).

Nominal Treatment Level (µg/kg)	Average Percent Survival (SD[a])	Average Dry Weight/Amphipod (SD) (mg)	Average Number of Offspring/Amphipod (SD)
Control	94 (3)	2.61 (0.28)	9.91 (8.67)
Solvent Control	95 (5)	2.64 (0.19)	14.17 (3.93)
Pooled Control	95 (4)	2.63 (0.23)	12.04 (6.74)
5.6	98 (4)	1.86 (0.33)[bc]	3.73 (2.24)[c]
17	98 (3)	2.76 (0.24)	4.61 (2.40)[c]
50	96 (7)	2.35 (0.28)	2.57 (2.21)[c]
150	75 (11)[c]	1.41 (0.17)[c]	0.53 (0.33)[c]
450	0 (0)[c]	NA[d]	NA[d]
1350	0 (0)[c]	NA	NA

[a] SD = Standard Deviation.
[b] Statistical reduction not considered biologically relevant due to lack of similar significance at the next two higher treatment levels.
[c] Statistically different (≤ 0.05) compared to the pooled control data.
[d] NA = Not Applicable.

OPPTS 850.1780 Chronic Sediment Leptocheirus **Springborn Smithers**

Analysis ID:	18-1653-7358	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.9.1
Analyzed:	19 Jul-16 13:24	**Analysis:**	Nonparametric-Two Sample	**Official Results:**	Yes
Batch ID:	16-7638-8390	**Test Type:**	Chronic Sediment Leptocheirus	**Analyst:**	J. Housenger
Start Date:	03 Feb-05	**Protocol:**	OPPTS 850.1780 Chronic Sediment Leptoc	**Diluent:**	
Ending Date:	03 Mar-05	**Species:**	Leptocheirus plumulosus	**Brine:**	
Duration:	28d 0h	**Source:**		**Age:**	
Sample ID:	09-3842-9590	**Code:**	46591501	**Client:**	EPA OCSPP EFED
Sample Date:	03 Feb-05	**Material:**	Bifenthrin	**Project:**	
Receipt Date:		**Source:**	Pyrethroid Working Group		
Sample Age:	n/a	**Station:**			

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	17	50	29.15		50.52%

Mann-Whitney U Two-Sample Test

Control	vs	Conc-µg/kg s	Test Stat	Critical	Ties	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		5.6	19	n/a	0	8	Exact	0.1032	Non-Significant Effect
		17	16.5	n/a	1	8	Exact	0.2262	Non-Significant Effect
		50*	21	n/a	0	8	Exact	0.0476	Significant Effect
		150*	25	n/a	0	8	Exact	0.0040	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	246.292	61.573	4	3.391	0.0285	Significant Effect
Error	363.168	18.1584	20			
Total	609.46		24			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance Test	27.13	13.28	1.9E-05	Unequal Variances
Distr bution	Shapiro-Wilk W Normality Test	0.8892	0.8877	0.0107	Normal Distribution

Reproduction Summary

Conc-µg/kg sed	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	5	9.92	-0.8343	20.67	5.7	2.2	22.6	3.873	87.31%	0.00%
5.6		5	3.74	0.9752	6.505	3.6	1.8	7.2	0.9958	59.54%	62.30%
17		5	4.64	1.662	7.618	4.6	1.5	8.2	1.073	51.69%	53.23%
50		5	2.58	-0.1845	5.344	1.2	1	6.1	0.9957	86.30%	73.99%
150		5	0.52	0.1138	0.9262	0.4	0.3	1.1	0.1463	62.91%	94.76%

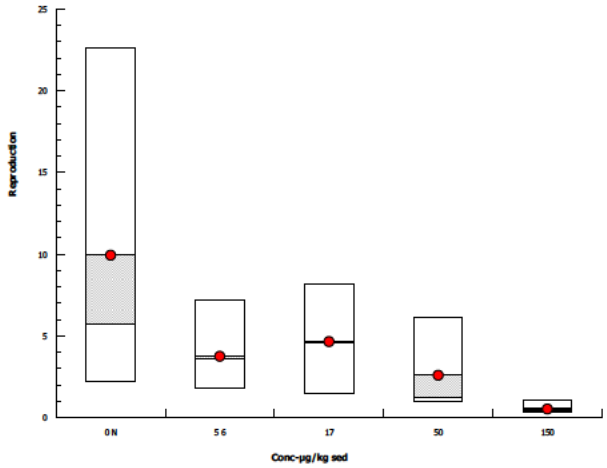
Reproduction Detail

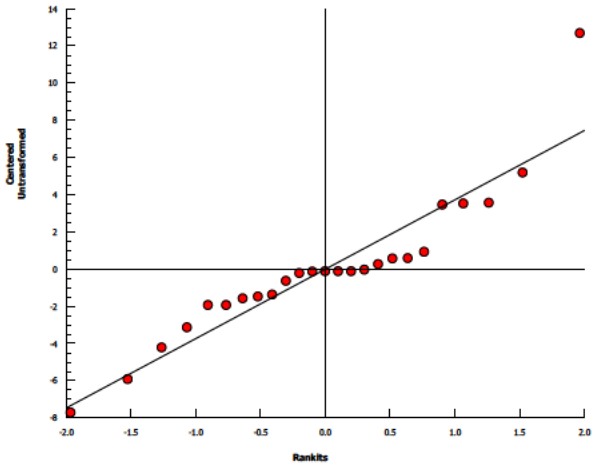
Conc-µg/kg sed	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	N	2.2	4	15.1	22.6	5.7
5.6		4.3	3.6	1.8	1.8	7.2
17		8.2	1.5	4.9	4.6	4
50		1.1	1	3.5	6.1	1.2
150		0.3	0.4	0.4	0.4	1.1

OPPTS 850.1780 Chronic Sediment Leptocheirus — **Springborn Smithers**

Analysis ID:	18-1653-7358	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.9.1
Analyzed:	19 Jul-16 13:24	**Analysis:**	Nonparametric-Two Sample	**Official Results:**	Yes

Graphics





OPPTS 850.1780 Chronic Sediment Leptocheirus **Springborn Smithers**

Analysis ID:	12-0277-5906	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.9.1
Analyzed:	19 Jul-16 13:24	**Analysis:**	Nonparametric-Control vs Ord. Treatments	**Official Results:**	Yes
Batch ID:	16-7638-8390	**Test Type:**	Chronic Sediment Leptocheirus	**Analyst:**	J. Housenger
Start Date:	03 Feb-05	**Protocol:**	OPPTS 850.1780 Chronic Sediment Leptoc	**Diluent:**	
Ending Date:	03 Mar-05	**Species:**	Leptocheirus plumulosus	**Brine:**	
Duration:	28d 0h	**Source:**		**Age:**	
Sample ID:	09-3842-9590	**Code:**	46591501	**Client:**	EPA OCSPP EFED
Sample Date:	03 Feb-05	**Material:**	Bifenthrin	**Project:**	
Receipt Date:		**Source:**	Pyrethroid Working Group		
Sample Age:	n/a	**Station:**			

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU
Untransformed	C > T	17	50	29.15	

Jonckheere-Terpstra Step-Down Test

Control vs	Conc-µg/kg s	Test Stat	Critical	Ties	P-Type	P-Value	Decision(α:5%)
Negative Control	5.6	19	n/a		Exact	0.2293	Non-Significant Effect
	17	0.7411	1.645	2	Asymp	0.2293	Non-Significant Effect
	50*	1.987	1.645	2	Asymp	0.0235	Significant Effect
	150*	3.735	1.645	4	Asymp	9.4E-05	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	246.292	61.573	4	3.391	0.0285	Significant Effect
Error	363.168	18.1584	20			
Total	609.46		24			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance Test	27.13	13.28	1.9E-05	Unequal Variances
Distr bution	Shapiro-Wilk W Normality Test	0.8892	0.8877	0.0107	Normal Distribution

Reproduction Summary

Conc-µg/kg sed	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	5	9.92	-0.8343	20.67	5.7	2.2	22.6	3.873	87.31%	0.00%
5.6		5	3.74	0.9752	6.505	3.6	1.8	7.2	0.9958	59.54%	62.30%
17		5	4.64	1.662	7.618	4.6	1.5	8.2	1.073	51.69%	53.23%
50		5	2.58	-0.1845	5.344	1.2	1	6.1	0.9957	86.30%	73.99%
150		5	0.52	0.1138	0.9262	0.4	0.3	1.1	0.1463	62.91%	94.76%

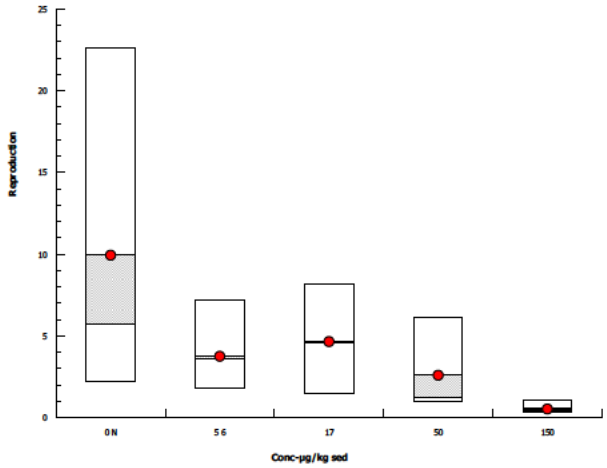
Reproduction Detail

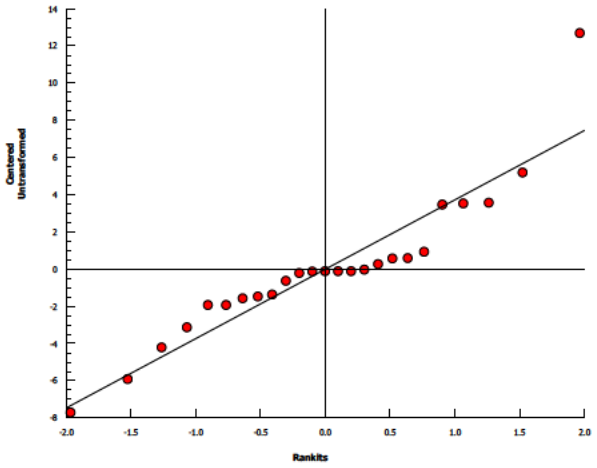
Conc-µg/kg sed	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	N	2.2	4	15.1	22.6	5.7
5.6		4.3	3.6	1.8	1.8	7.2
17		8.2	1.5	4.9	4.6	4
50		1.1	1	3.5	6.1	1.2
150		0.3	0.4	0.4	0.4	1.1

OPPTS 850.1780 Chronic Sediment Leptocheirus — **Springborn Smithers**

Analysis ID:	12-0277-5906	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.9.1
Analyzed:	19 Jul-16 13:24	**Analysis:**	Nonparametric-Control vs Ord. Treatments	**Official Results:**	Yes

Graphics





OPPTS 850.1780 Chronic Sediment Leptocheirus **Springborn Smithers**

Analysis ID:	08-6934-2260	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.9.1
Analyzed:	19 Jul-16 13:26	**Analysis:**	Nonparametric-Two Sample	**Official Results:**	Yes
Batch ID:	16-7638-8390	**Test Type:**	Chronic Sediment Leptocheirus	**Analyst:**	J. Housenger
Start Date:	03 Feb-05	**Protocol:**	OPPTS 850.1780 Chronic Sediment Leptoc	**Diluent:**	
Ending Date:	03 Mar-05	**Species:**	Leptocheirus plumulosus	**Brine:**	
Duration:	28d 0h	**Source:**		**Age:**	
Sample ID:	09-3842-9590	**Code:**	46591501	**Client:**	EPA OCSPP EFED
Sample Date:	03 Feb-05	**Material:**	Bifenthrin	**Project:**	
Receipt Date:		**Source:**	Pyrethroid Working Group		
Sample Age:	n/a	**Station:**			

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	< 5.6	5.6	n/a		34.92%

Mann-Whitney U Two-Sample Test

Control	vs	Control II	Test Stat	Critical	Ties	DF	P-Type	P-Value	Decision(α:5%)
Pooled Controls		5.6*	44	n/a	0	13	Exact	0.0097	Significant Effect
		17*	41.5	n/a	1	13	Exact	0.0203	Significant Effect
		50*	46	n/a	0	13	Exact	0.0040	Significant Effect
		150*	50	n/a	0	13	Exact	3.3E-04	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	609.298	152.324	4	8.096	2.5E-04	Significant Effect
Error	470.365	18.8146	25			
Total	1079.66		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance Test	25.88	13.28	3.4E-05	Unequal Variances
Distr bution	Shapiro-Wilk W Normality Test	0.9257	0.9031	0.0378	Normal Distribution

Reproduction Summary

Conc-µg/kg sed	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	@	10	12.05	7.238	16.86	12.9	2.2	22.6	2.127	55.83%	0.00%
5.6		5	3.74	0.9752	6.505	3.6	1.8	7.2	0.9958	59.54%	68.96%
17		5	4.64	1.662	7.618	4.6	1.5	8.2	1.073	51.69%	61.49%
50		5	2.58	-0.1845	5.344	1.2	1	6.1	0.9957	86.30%	78.59%
150		5	0.52	0.1138	0.9262	0.4	0.3	1.1	0.1463	62.91%	95.68%

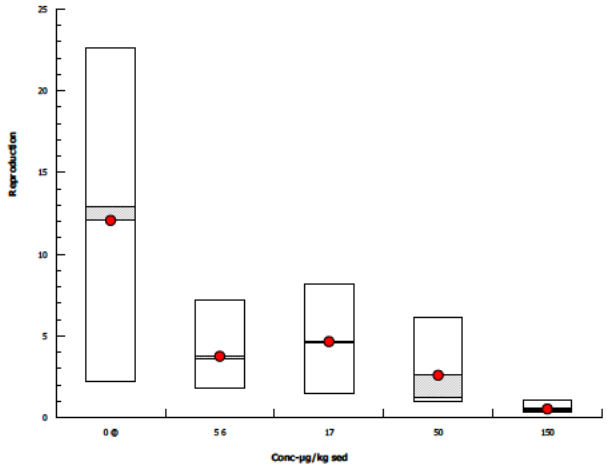
Reproduction Detail

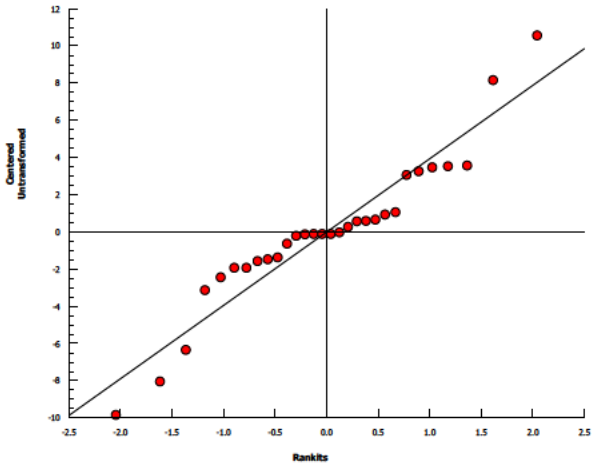
Conc-µg/kg sed	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	@	9.6	20.2	12.7	13.1	15.3	2.2	4	15.1	22.6	5.7
5.6		4.3	3.6	1.8	1.8	7.2					
17		8.2	1.5	4.9	4.6	4					
50		1.1	1	3.5	6.1	1.2					
150		0.3	0.4	0.4	0.4	1.1					

OPPTS 850.1780 Chronic Sediment Leptocheirus — **Springborn Smithers**

Analysis ID:	08-6934-2260	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.9.1
Analyzed:	19 Jul-16 13:26	**Analysis:**	Nonparametric-Two Sample	**Official Results:**	Yes

Graphics





OPPTS 850.1780 Chronic Sediment Leptocheirus **Springborn Smithers**

Analysis ID:	15-7656-2784	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.9.1
Analyzed:	19 Jul-16 13:26	**Analysis:**	Nonparametric-Control vs Ord. Treatments	**Official Results:**	Yes
Batch ID:	16-7638-8390	**Test Type:**	Chronic Sediment Leptocheirus	**Analyst:**	J. Housenger
Start Date:	03 Feb-05	**Protocol:**	OPPTS 850.1780 Chronic Sediment Leptoc	**Diluent:**	
Ending Date:	03 Mar-05	**Species:**	Leptocheirus plumulosus	**Brine:**	
Duration:	28d 0h	**Source:**		**Age:**	
Sample ID:	09-3842-9590	**Code:**	46591501	**Client:**	EPA OCSPP EFED
Sample Date:	03 Feb-05	**Material:**	Bifenthrin	**Project:**	
Receipt Date:		**Source:**	Pyrethroid Working Group		
Sample Age:	n/a	**Station:**			

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU
Untransformed	C > T	< 5.6	5.6	n/a	

Jonckheere-Terpstra Step-Down Test

Control vs	Control II	Test Stat	Critical	Ties	P-Type	P-Value	Decision(α:5%)
Pooled Controls	5.6*	44	n/a		Exact	0.0097	Significant Effect
	17*	2.277	1.645	2	Asymp	0.0114	Significant Effect
	50*	3.292	1.645	2	Asymp	5.0E-04	Significant Effect
	150*	4.718	1.645	4	Asymp	1.2E-06	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	609.298	152.324	4	8.096	2.5E-04	Significant Effect
Error	470.365	18.8146	25			
Total	1079.66		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance Test	25.88	13.28	3.4E-05	Unequal Variances
Distr bution	Shapiro-Wilk W Normality Test	0.9257	0.9031	0.0378	Normal Distribution

Reproduction Summary

Conc-µg/kg sed	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	@	10	12.05	7.238	16.86	12.9	2.2	22.6	2.127	55.83%	0.00%
5.6		5	3.74	0.9752	6.505	3.6	1.8	7.2	0.9958	59.54%	68.96%
17		5	4.64	1.662	7.618	4.6	1.5	8.2	1.073	51.69%	61.49%
50		5	2.58	-0.1845	5.344	1.2	1	6.1	0.9957	86.30%	78.59%
150		5	0.52	0.1138	0.9262	0.4	0.3	1.1	0.1463	62.91%	95.68%

Reproduction Detail

Conc-µg/kg sed	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	@	9.6	20.2	12.7	13.1	15.3	2.2	4	15.1	22.6	5.7
5.6		4.3	3.6	1.8	1.8	7.2					
17		8.2	1.5	4.9	4.6	4					
50		1.1	1	3.5	6.1	1.2					
150		0.3	0.4	0.4	0.4	1.1					

OPPTS 850.1780 Chronic Sediment Leptocheirus — **Springborn Smithers**

Analysis ID:	15-7656-2784	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.9.1
Analyzed:	19 Jul-16 13:26	**Analysis:**	Nonparametric-Control vs Ord. Treatments	**Official Results:**	Yes

Graphics